

Discussion Letter

A cross-linking mechanism for the heparin-enhanced antithrombin–thrombin reaction?

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Emerging evidence suggests that a simple cross-linking reaction through ionic interaction may be the primary cause of the heparin-accelerated antithrombin inhibition of α -thrombin.

Heparin Antithrombin Thrombin

1. INTRODUCTION

The molecular mechanism of heparin-enhanced antithrombin–thrombin reaction [1–4] is a debated subject. Three models are currently available to account for the action of heparin. (i) The heparin–antithrombin model [5–7] postulates that heparin binds to antithrombin and transforms antithrombin into a more effective inhibitor. (ii) The heparin–thrombin model [8–11] postulates that heparin binds to thrombin and makes thrombin more susceptible to antithrombin inhibition. (iii) The ternary (template) model [12–17] postulates that heparin must interact with both antithrombin and thrombin to enhance the inhibition. The fact that all 3 models are well sustained by experimental evidence suggests that a compromised working model must accommodate all experimental results supporting each of those models.

There is now growing evidence to indicate that

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Thrombin refers to α -thrombin unless otherwise indicated

the ternary model [12] may represent the compromise between the heparin–thrombin and heparin–antithrombin models as the practical working model. This evidence further implies that heparin accelerates the formation of the antithrombin–thrombin complex by acting primarily as a noncovalent cross-linking reagent, and that the acidic sulfate groups of heparin cross-link antithrombin and thrombin by interacting with the basic cross-linking sites of antithrombin and thrombin independently.

This evidence, derived from both chemical and kinetic studies, may be summarized as follows:

(i) It is now well demonstrated that heparin reacts with antithrombin [3,18,19] and thrombin [9,11,20–23] independently. The reactions require the acidic sulfate groups of heparin [16,24–26] and the basic amino acids (Lys and Arg) of both antithrombin [3,12,27,30] and thrombin [8,10,12,28]. In addition to heparin, sulfate polymers such as polyanetholesulfonate [27,29], dermatan sulfate [31,32], heparan sulfate [32] and pentosane polysulfate [33,34] also display heparin-like activity in enhancing antithrombin–thrombin reaction.

(ii) Chemical studies [8,10,12] have shown that the

heparin binding sites of antithrombin and thrombin are independent of their reactive site and active site, respectively. The sensitivity of heparin-enhanced antithrombin-thrombin reaction can be diminished or abolished by masking the heparin-binding site (through chemical modification of Lys and Arg) of antithrombin [3,12] and/or thrombin [8,10,12,28]. On the other hand, blocking of the active site of thrombin does not prevent heparin-mediated cross-linking of antithrombin and thrombin [12].

(iii) The most convincing evidence for the cross-linking mechanism comes from kinetic studies which have shown that interactions of both antithrombin and thrombin with heparin are involved in the expression of activity [14,17]. At fixed concentrations of antithrombin and thrombin, the reaction rates of antithrombin and thrombin always display a bell-shaped curve against the increase of heparin concentration [6,14,17,35–37]; i.e., there is an optimal heparin concentration for each reaction mixture with fixed concentrations of antithrombin and thrombin. The molar ratios of antithrombin/heparin/thrombin observed under those optimal conditions are strikingly close to 1:1:1 [14]. These results are characteristic of protein cross-linking phenomena: at low concentrations of heparin, both antithrombin and thrombin will compete for binding to heparin; at excess concentrations of heparin, both antithrombin and thrombin will be saturated with heparin; and the optimal cross-linking reaction occurs when the cross-linking reagent and proteins have approximately equal concentrations.

Finally, the binding mechanism of this proposed cross-linking model bears an interesting resemblance to the binding mechanism of the hirudin-thrombin complex [38]. Efficient inhibition of both hirudin and antithrombin requires interaction with α -thrombin through the active site as well as an independent basic site [39,40]. This resemblance, together with the intriguing sequence homology of hirudin and fibrinopeptides [38], suggests that a common mechanism may account for the unique enzymatic specificity of α -thrombin toward both protein inhibitors and protein substrates.

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